UK NEQAS for Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 141503**

Date Issued: 26-January-2015 **Closing Date: 13-February-2015**

Your Results

Sample: 073

Trial Comments

This trial was issued to 159 participants worldwide for the identification of PNH clones

Sample Comment
The sample was manufactured by UK NEQAS using a stabilised whole blood unit

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		0.00	0.00	0.00	121
RBC Type II Cells - CD59		0.00	0.00	0.00	112
RBC Type III Cells - CD59		0.00	0.00	0.00	113
Granulocytes PNH Clone - CD16		0.00	0.00	0.00	32
Granulocytes PNH Clone - CD24		0.00	0.00	0.00	102
Granulocytes PNH Clone - CD55		0.00	0.00	0.10	17
Granulocytes PNH Clone - CD59		0.00	0.00	0.00	15
Granulocytes PNH Clone - CD66b		0.00	0.00	0.00	28
Granulocytes PNH Clone - FLAER		0.00	0.00	0.00	124

Performance Monitoring

Sample 073

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Absent		Outwith Consensus	
WBC	Clone Absent		Outwith Consensus	

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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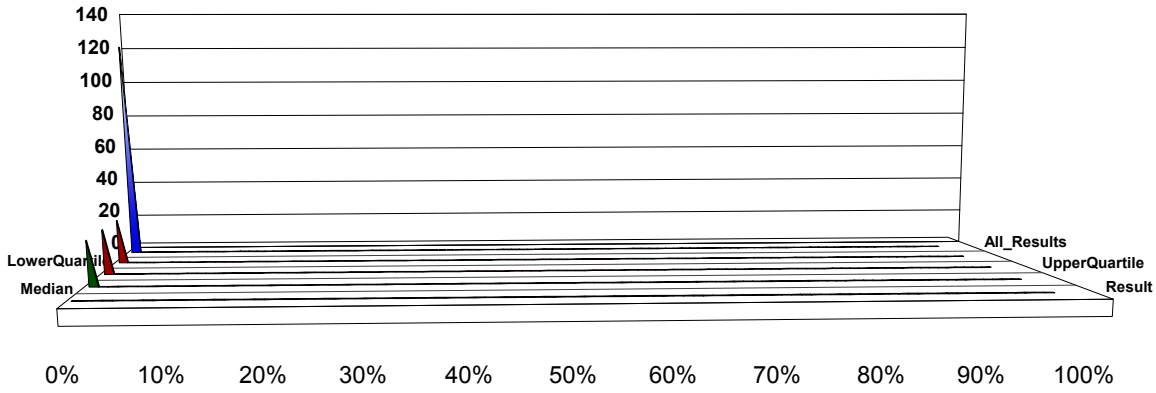
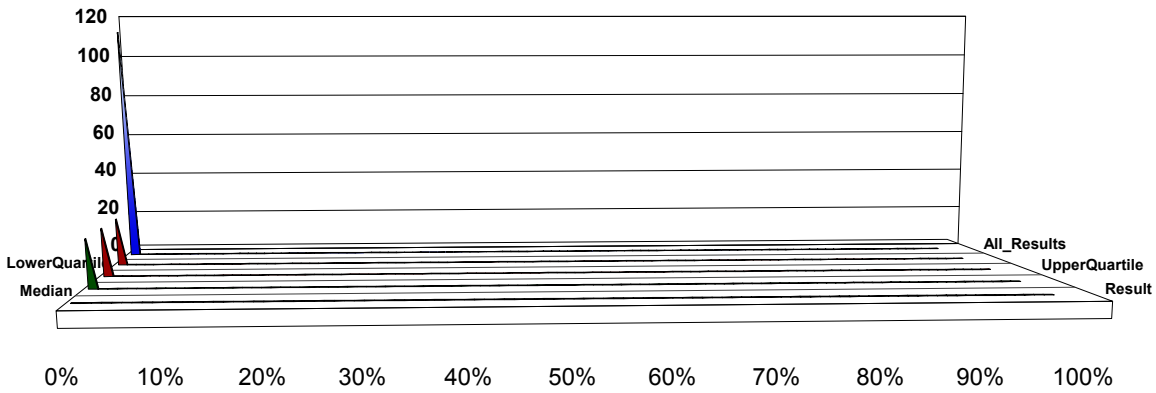
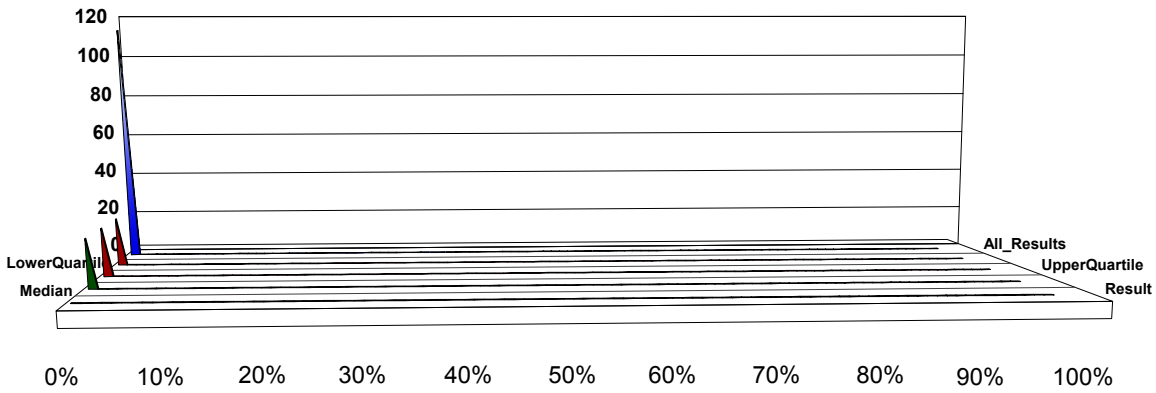
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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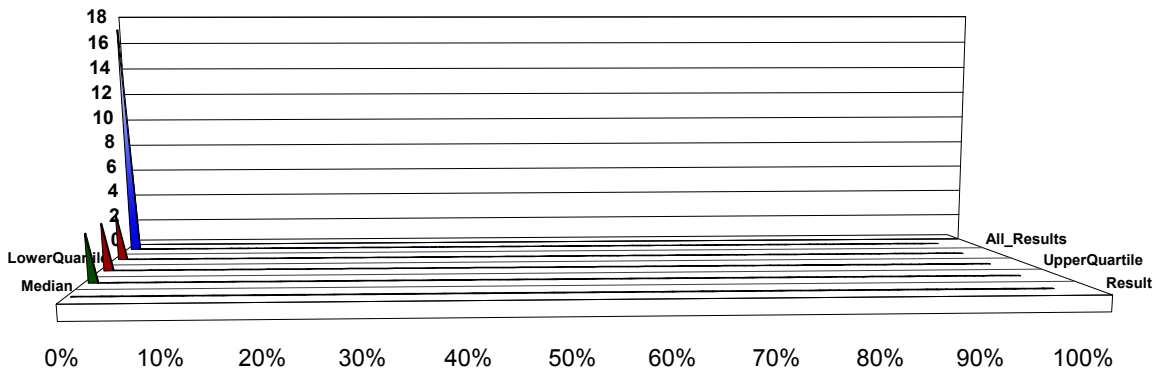
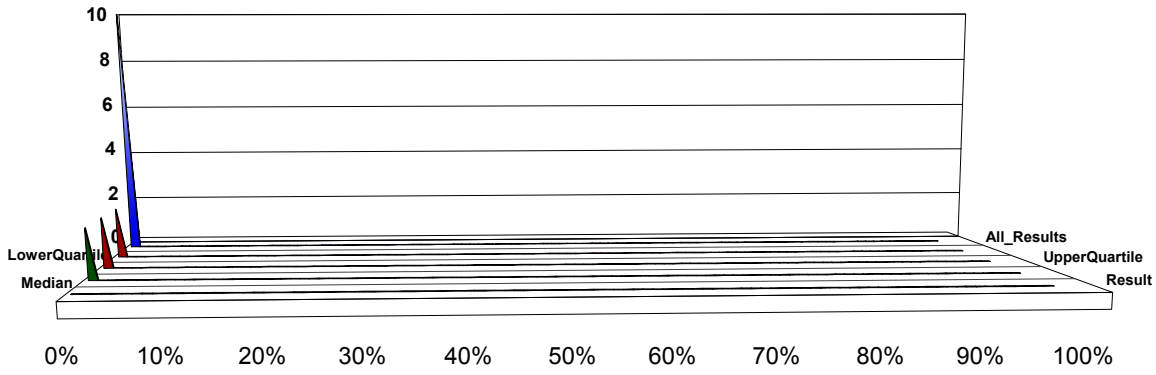
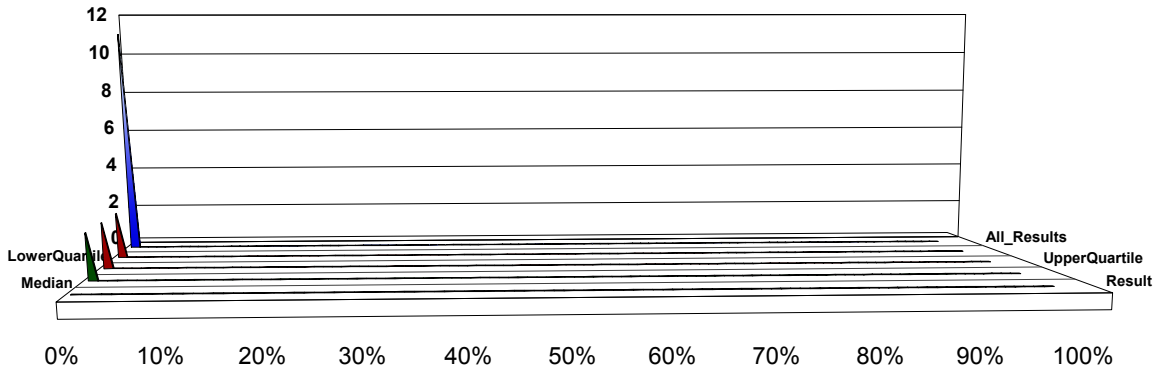
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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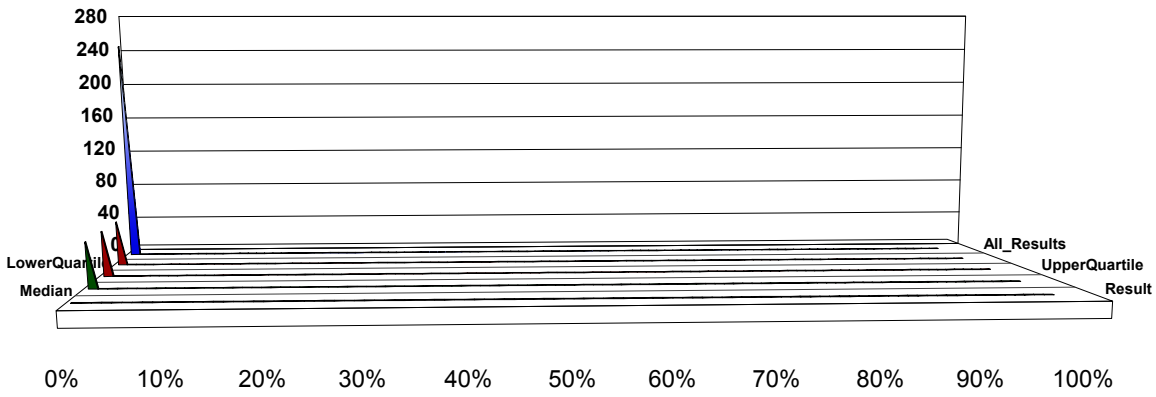
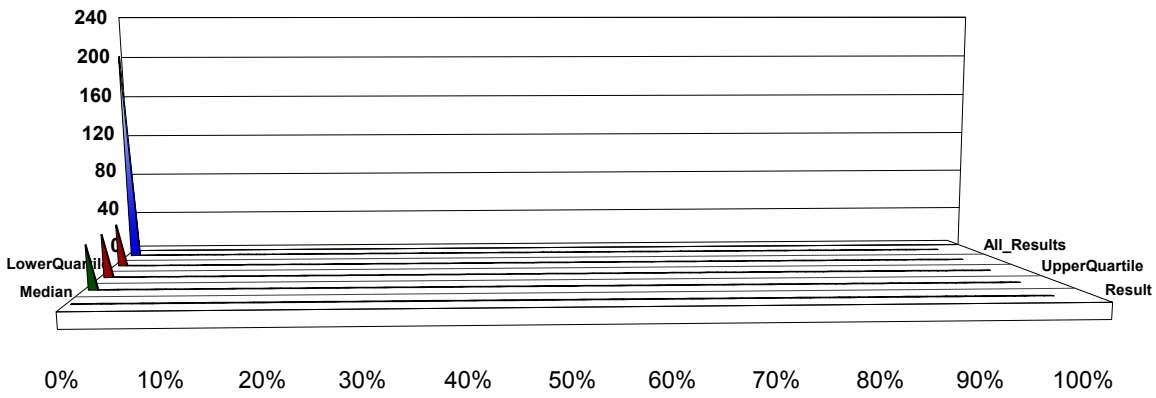
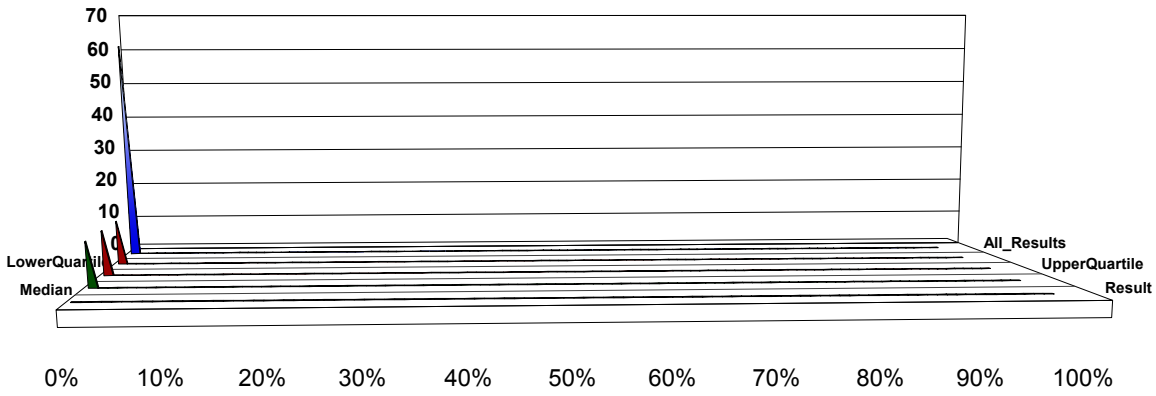
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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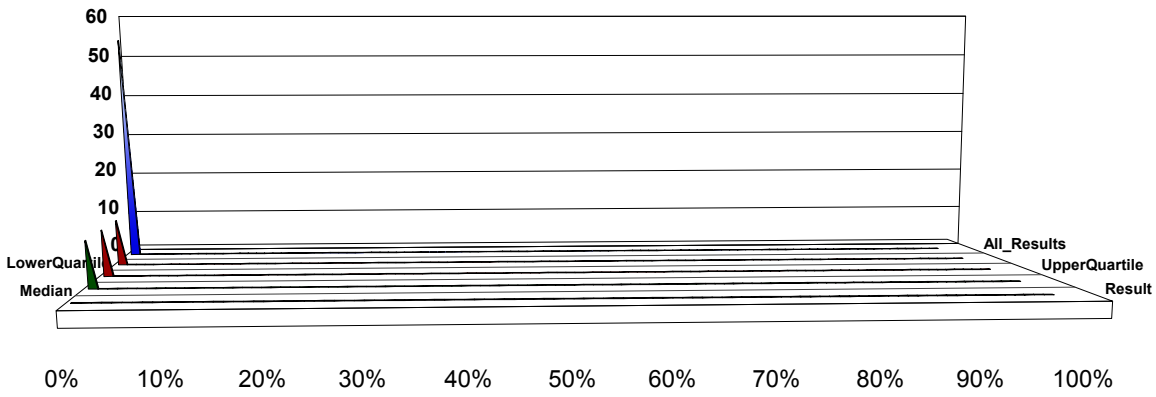
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.01	17
BD	0.00	0.00	0.00	14
CD59				
All	0.00	0.00	0.00	121
BD	0.00	0.00	0.00	86
Coulter	0.00	0.00	0.00	34

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.01	17
BD Biosciences	0.00	0.00	0.08	10
CD59				
All	0.00	0.00	0.00	121
BD Biosciences	0.00	0.00	0.03	16
invitrogen	0.00	0.00	0.00	64

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.00	0.00	0.01	17
PE	0.00	0.00	0.08	14
CD59				
All	0.00	0.00	0.00	121
PE	0.00	0.00	0.00	94
FITC	0.00	0.00	0.01	26

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	32
BD	0.00	0.00	0.00	24
CD24				
All	0.00	0.00	0.00	102
BD	0.00	0.00	0.00	71
Coulter	0.00	0.00	0.00	30
CD55				
All	0.00	0.00	0.10	17
BD	0.00	0.00	0.13	10
CD59				
All	0.00	0.00	0.00	15
BD	0.00	0.00	0.08	10
CD66b				
All	0.00	0.00	0.00	28
BD	0.00	0.00	0.00	20
FLAER				
All	0.00	0.00	0.00	124
BD	0.00	0.00	0.00	85
Coulter	0.00	0.00	0.00	38

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	32
BD Biosciences	0.00	0.00	0.00	12
Beckman Coulter	0.00	0.00	0.00	11
CD24				
All	0.00	0.00	0.00	102
BD Biosciences	0.00	0.00	0.00	31
Beckman Coulter	0.00	0.00	0.00	47
Pharmingen	0.00	0.00	0.00	18
CD55				
All	0.00	0.00	0.10	17
CD59				
All	0.00	0.00	0.00	15
CD66b				
All	0.00	0.00	0.00	28
BD Biosciences	0.00	0.00	0.00	12
FLAER				
All	0.00	0.00	0.00	124
Other	0.00	0.00	0.00	65
Protox	0.00	0.00	0.00	50

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	0.00	0.00	0.00	32
CD24				
All	0.00	0.00	0.00	102
PE	0.00	0.00	0.00	76
CD55				
All	0.00	0.00	0.10	17
PE	0.00	0.00	0.11	12
CD59				
All	0.00	0.00	0.00	15
PE	0.00	0.00	0.00	10
CD66b				
All	0.00	0.00	0.00	28
FITC	0.00	0.00	0.00	22
FLAER				
All	0.00	0.00	0.00	124
FITC	0.00	0.00	0.00	38
AlexaFluor 488	0.00	0.00	0.00	85

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Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 141503**

Date Issued: 26-January-2015 **Closing Date: 13-February-2015**

Your Results

Sample: 074

Trial Comments

This trial was issued to 159 participants worldwide for the identification of PNH clones

Sample Comment
The sample was manufactured by UK NEQAS using a sample of blood from a PNH patient and a stabilised whole blood unit

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		0.60	0.33	1.90	121
RBC Type II Cells - CD59		0.36	0.13	1.60	113
RBC Type III Cells - CD59		0.20	0.18	0.30	113
Granulocytes PNH Clone - CD16		15.63	14.52	17.48	32
Granulocytes PNH Clone - CD24		15.40	13.60	17.75	103
Granulocytes PNH Clone - CD55		15.50	12.70	17.00	17
Granulocytes PNH Clone - CD59		17.76	9.60	20.05	15
Granulocytes PNH Clone - CD66b		15.60	14.25	18.00	29
Granulocytes PNH Clone - FLAER		15.40	13.60	17.42	125

Performance Monitoring

Sample 074

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Present		Outwith Consensus	
WBC	Clone Present		Outwith Consensus	

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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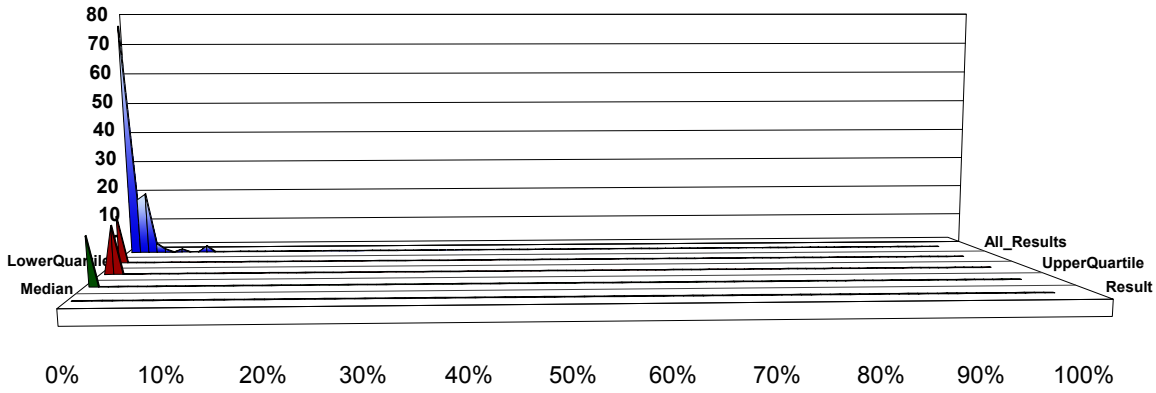
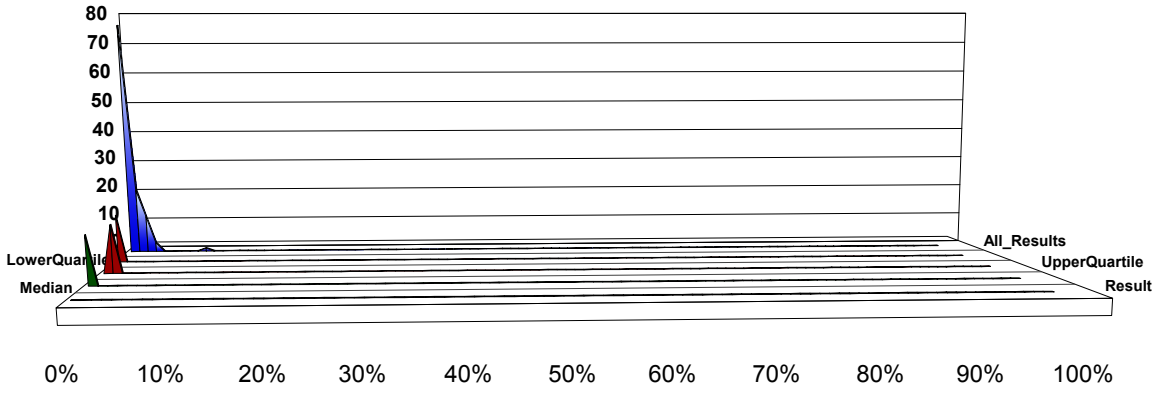
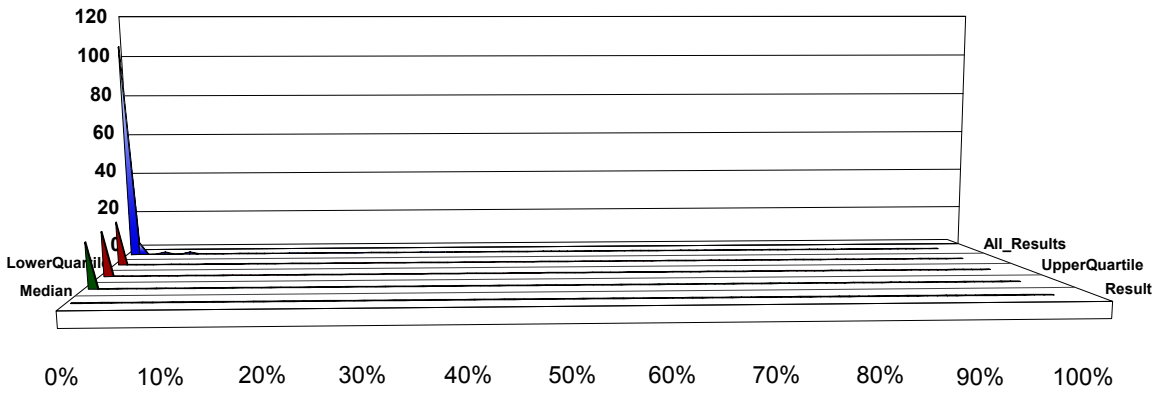
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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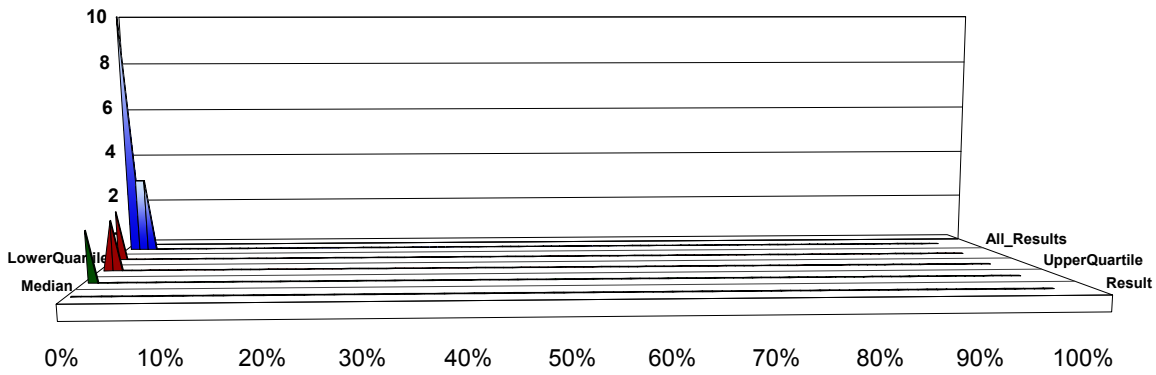
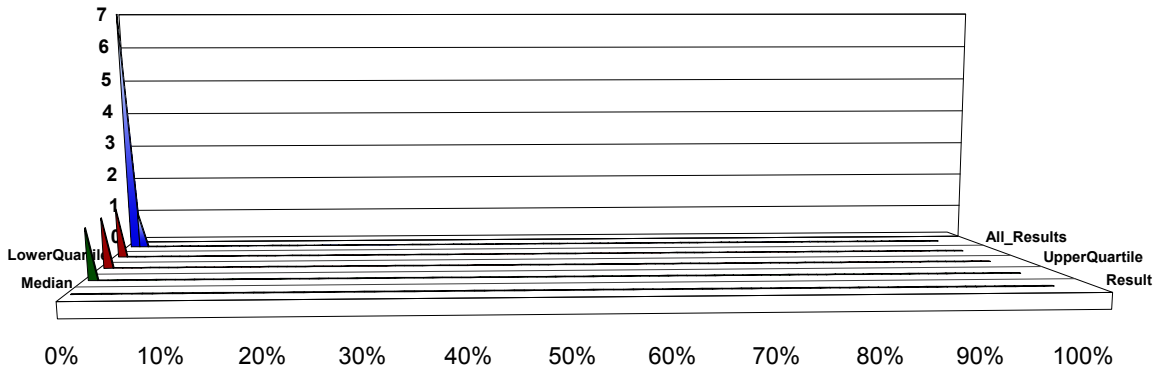
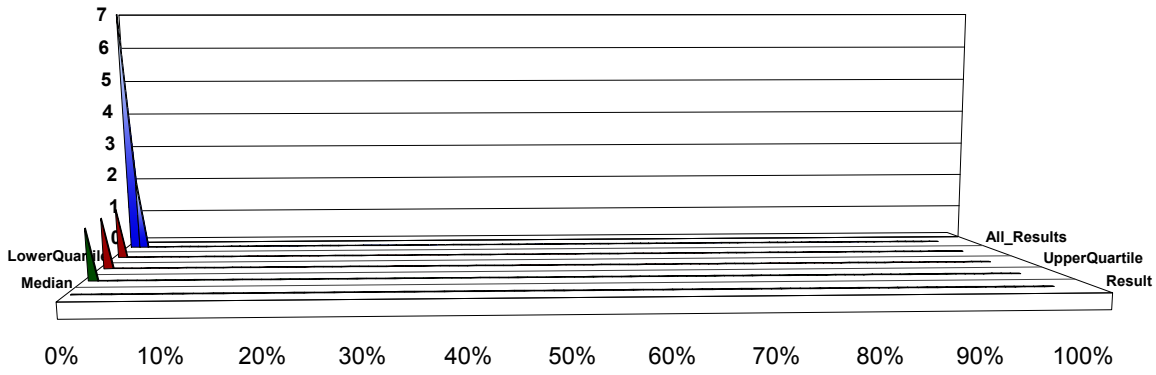
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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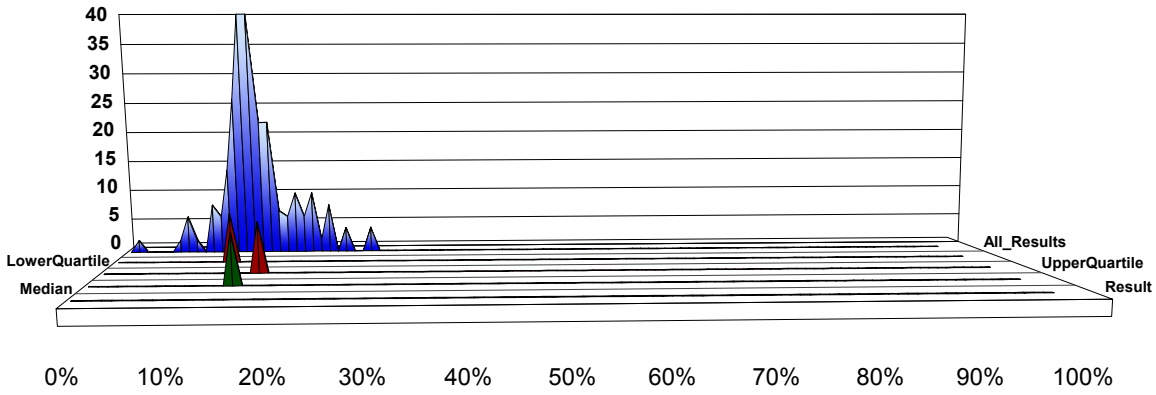
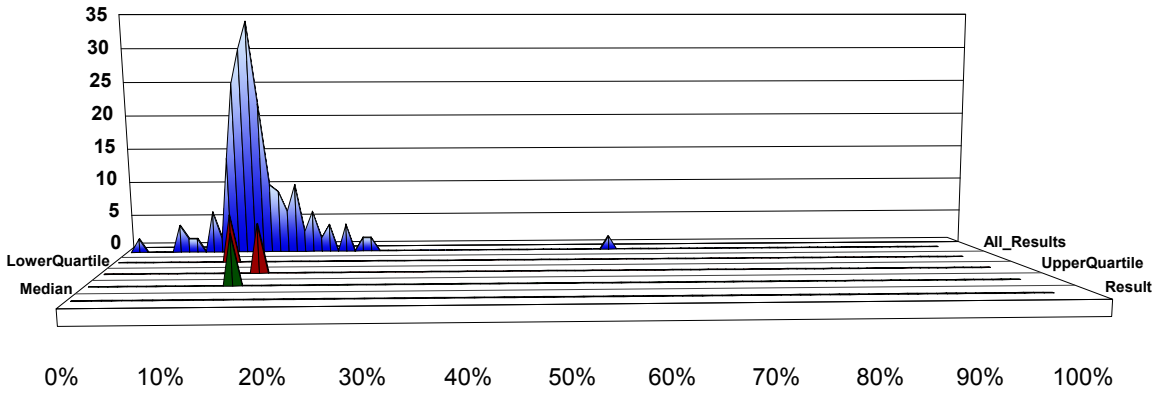
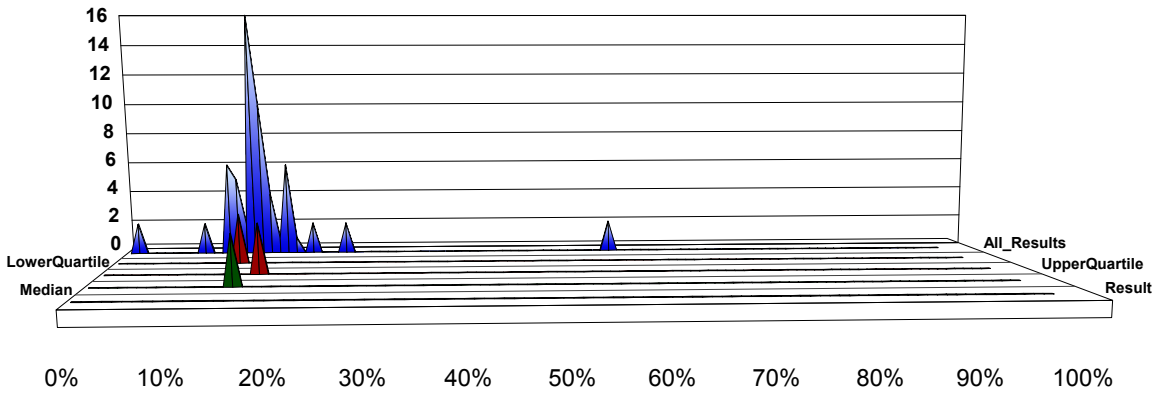
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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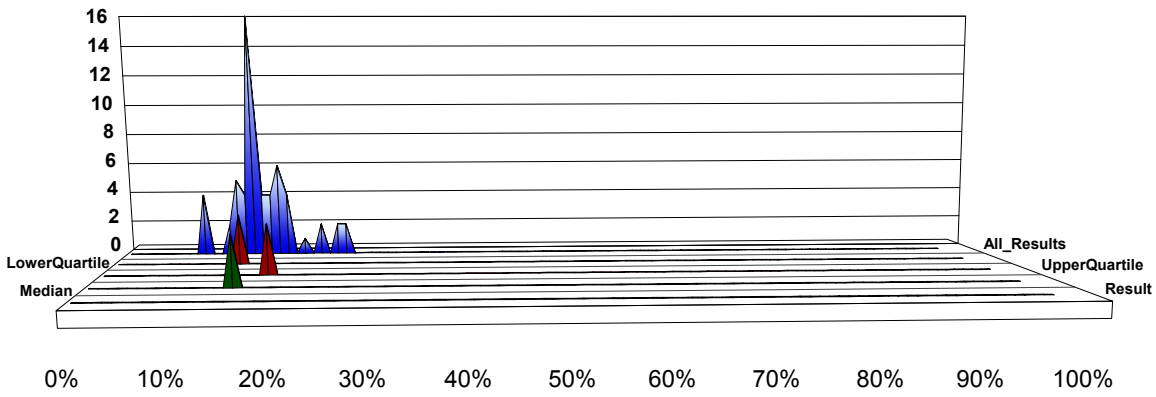
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.55	0.30	1.51	16
BD	0.60	0.35	1.41	13
CD59				
All	0.60	0.33	1.90	121
BD	0.58	0.33	1.91	85
Coulter	0.60	0.32	1.77	35

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.55	0.30	1.51	16
CD59				
All	0.60	0.33	1.90	121
BD Biosciences	0.80	0.11	2.09	15
invitrogen	0.70	0.40	1.74	65

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.55	0.30	1.51	16
PE	0.65	0.36	1.70	14
CD59				
All	0.60	0.33	1.90	121
PE	0.60	0.36	1.79	94
FITC	0.40	0.30	1.97	26

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	15.63	14.52	17.48	32
BD	16.15	15.00	17.48	24
CD24				
All	15.40	13.60	17.75	103
BD	15.40	14.06	17.00	71
Coulter	14.86	12.35	20.12	31
CD55				
All	15.50	12.70	17.00	17
BD	15.90	15.13	17.90	10
CD59				
All	17.76	9.60	20.05	15
BD	17.33	7.95	20.33	10
CD66b				
All	15.60	14.25	18.00	29
BD	15.65	15.15	18.31	20
FLAER				
All	15.40	13.60	17.42	125
BD	15.40	14.01	17.13	85
Coulter	15.00	12.65	18.76	39

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	15.63	14.52	17.48	32
BD Biosciences	15.14	14.52	16.63	12
Beckman Coulter	15.40	12.60	18.28	11
CD24				
All	15.40	13.60	17.75	103
BD Biosciences	15.28	13.44	17.32	31
Beckman Coulter	15.55	13.55	19.86	48
Pharmingen	14.70	14.00	16.00	18
CD55				
All	15.50	12.70	17.00	17
CD59				
All	17.76	9.60	20.05	15
CD66b				
All	15.60	14.25	18.00	29
BD Biosciences	15.60	13.24	17.25	12
FLAER				
All	15.40	13.60	17.42	125
Other	15.00	13.00	17.10	65
Protox	15.96	14.40	20.17	50

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	15.63	14.52	17.48	32
CD24				
All	15.40	13.60	17.75	103
PE	15.24	13.66	16.70	76
CD55				
All	15.50	12.70	17.00	17
PE	14.20	6.28	18.65	12
CD59				
All	17.76	9.60	20.05	15
PE	18.32	16.23	21.29	10
CD66b				
All	15.60	14.25	18.00	29
FITC	15.41	14.14	17.75	22
FLAER				
All	15.40	13.60	17.42	125
FITC	15.35	14.24	18.09	40
AlexaFluor 488	15.40	13.60	17.20	85

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PNH 141503

Sample 073

This sample was normal whole blood containing no PNH clone; the median values of the results returned reflected those obtained at pre-issue testing.

The consensus result for this sample was **Clone Absent** for both Red Cell and Granulocyte clones. 20 participants recorded the presence of a small red cell clone (0.008%-0.38%); however 19 of these were then reported as clone absent, the other 1 centre reported their result as clone present (clone size 0.79%). 16 participants recorded a small granulocyte clone a clone on at least one of their markers (clone size 0.003%-0.38%) all were reported as being clone absent.

Sample 074

The median values of the results returned for this sample reflected those obtained at pre-issue testing.

The consensus result for this sample was **Clone Present** for both Red Cell and Granulocyte clones. 12 participants were out of consensus for red cell analysis, with 8 failing to identify the clone altogether; four centres identified a clone (clone size 0.3%-0.9%) and reported the results as clone absent. Only 1 participant was out of consensus with regards to granulocyte clones and reported clone absent having identified a 0.8% clone using CD14. CD14 is not suitable for the detection of granulocyte PNH clones. Whilst CD14 is expressed on monocytes and may be used to detect a PNH monocyte clone, at present we do not ask for monocyte clones and we kindly request therefore that those participants using CD14 on monocytes do not report these results as granulocyte clones.

11 Participants failed to return any results for both samples.

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance. Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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